ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF
2-(4-FLUOROBENZYLTHIO)-N-(SUBSTITUTED PHENYL) PYRIMIDINE-4-AMINES

NM. Goudgaon* and Rohini Yerram Reddy
Department of Post Graduate Studies and Research in Chemistry, Gulbarga University, Gulbarga – 585 106, Karnataka, India.

ABSTRACT
Pyrimidine scaffold being an integral part of DNA and RNA, occupy a unique and distinctive role in medicinal chemistry. In the past few years, fluorinated heterocyclic systems have been incorporated into drug discovery research to improve the drug physico-chemical properties. In view of this rational we have synthesized 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines (1a-k) and evaluated them for analgesic and anti-inflammatory activities. The analgesic and anti-inflammatory activities of the synthesized compounds 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines (1a-k) were carried out in albino mice and spruee dawley rats respectively. Compounds 1c and 1f showed good analgesic activity and remaining compounds exhibited moderate to poor analgesic activity when compared to the standard drug Pentazocin. Compounds 1a and 1j showed excellent anti-inflammatory activity and compounds 1g, 1h, 1i and 1k showed good anti-inflammatory activity in comparison with the standard drug Diclofenac sodium. Remaining compounds exhibited moderate to poor anti-inflammatory activity. The present study showed that fluorine substituted heterocycles might serve as pharmacologically potent and biologically important drugs.

Keywords: Analgesic activity, Anti-inflammatory activity, Pyrimidines.

INTRODUCTION
Pyrimidine scaffold being an integral part of DNA and RNA, occupy a unique and distinctive role in medicinal chemistry. Pyrimidine derivatives have been reported to possess a variety of pharmacological activities, notable among are the antibacterial, antihypertensive, antihistaminic, antifungal, anti-inflammatory, antiviral, and antitumor agents. In the past few years fluorinated heterocyclic systems have been incorporated into drug discovery research to improve the drug physico-chemical properties. Inflammation is a complex defensive mechanism of the body to any noxious stimulus; this process may vary from a localized to a generalized response characterized by the accumulation of fluids and leukocytes leading to edema and pain. Inflammation plays an important role in various diseases with high prevalence within population such as rheumatoid arthritis, atherosclerosis and asthma. During our drug discovery program, we have synthesized 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amines (1a-k) (Figure 1) and herein report the analgesic and anti-inflammatory activities.

![Fig. 1: Structure of Compounds (1a-k)](image-url)
2. MATERIALS AND METHODS
Adult healthy albino mice (18-24 gm) of either sex were used for analgesic activity. Male or female sprague dawley rats (150-180 gm) were used for anti-inflammatory activity. All the animals were maintained under controlled standard animal house conditions with easy access to food and water. The institutional ethical committee for animal cares and use approved for the experimental procedure (Reg. No: 1046/a/07/CPCSEA).

2.1. Hot plate method
Eddy’s hot plate method was used for the measurement of the analgesic activity by using Pentazocin as standard. In the hot plate method, albino mice were divided into ten groups with six animals in each group. One group served as a negative control (received 5% gum acacia 5 ml/kg), the second group received Pentazocin (5 mg/kg), while the third to tenth group received the compounds 1a, 1b, 1c, 1d, 1e, 1f, 1g and 1j respectively (200 mg/kg p.o.). Each mouse was placed on a hotplate (55°C) and the time lapse for the mouse to respond to the thermal pain (reaction time) was noted. Rubbing of palms or jumping was used as endpoint.

2.2. Mercury displacement method
Plethysmograph (mercury displacement method) was used for evaluation of anti-inflammatory activity against Diclofenac sodium as standard. For anti-inflammatory activity thirteen groups of six animals in each group were made serving for control (group-I), standard (group-II) and test compounds (group-III to XIII). Control groups received control, standard group received Diclofenac sodium 5 mg/kg p.o. and group-III to XIII received test compounds. Sixty minutes later rats were challenged with subcutaneous injection of 0.1 ml 1% formalin into the plantar region of left hind paw. The paw is marked at the level of lateral malleolus and immersed up to the mark. Paw volume is measured plethysmographically immediately after injection and again at 1, 2, 3 and 4 hour. The results were subjected for one way annova by using Graph pad prism software.

3. RESULT AND DISCUSSION
3.1. In-vivo analgesic activity
Analgesic activity was carried out by hot plate method by using Pentazocin as standard. Compounds 1c and 1f showed good analgesic activity and remaining compounds exhibited moderate to poor analgesic activity when compared to the standard drug Pentazocin. The results were subjected for one way annova by using Graph Pad Prism software. The results are tabulated in (Table 1) and shown graphically in (Figure 2).

3.2. In-vivo anti-inflammatory activity
The anti-inflammatory activity was studied by formalin induced rat hind paw oedema model measured by plethysmograph (mercury displacement method) using Diclofenac sodium as standard. Compounds 1a and 1j showed excellent anti-inflammatory activity, compounds 1g, 1h, 1i and 1k showed good anti-inflammatory activity in comparison with the standard drug Diclofenac sodium. Remaining compounds exhibited moderate to poor anti-inflammatory activity. The results were subjected for one way annova by using graph pad prism software. The effect of drugs in percentage reduction in paw volume and mean increase in paw volume are tabulated in (Table 2 & 3) and shown graphically in (Figure 3 & 4).

4. STRUCTURE ACTIVITY RELATIONSHIP OF COMPOUNDS (1a-k)
SAR of the compounds 2-(4-fluorobenzylthio)-N-(substituted phenyl) pyrimidine-4-amine (1a-k) has been investigated. Compounds 1c and 1f have shown good analgesic activity which may be due to the presence of a fluoro substituent at its para and meta position on the aromatic nucleus. Introduction of a fluoro substitution on the phenyl ring at the ortho (1a) position resulted in loss of potency. This indicates that small atom like fluoro which is similar to hydrogen in atomic radius is well tolerated in the meta and para positions, but substitution at the ortho position diminishes activity. The anti-inflammatory activity of 1a also may be due to the presence of fluoro substitution at its ortho position and the activity of 1j may be due to the presence of electron withdrawing nitro group at its para position which inhibits COX-2, playing an important role in inflammation and wound healing. The drug receptor interactions might be stronger through the hydrogen bonding between nitro group and receptors, while the activity decreased for the ortho (1b) and meta (1i) substituted nitro compounds due to intramolecular hydrogen bonding but activity was higher when compared to remaining compounds. Compound 1k also showed good activity may be because of additional halogen viz iodo in addition to fluoro at its para and ortho position and remaining compounds showed moderate to poor activity.

5. CONCLUSION
The present pharmacological studies of 2-(4-fluorobenzylthio)-N-(substituted phenyl)pyrimidine-4-amine (1a-k) indicates...
that introduction of electronegative fluorine on the aromatic nucleus led to the enhancement of biological activity. Further, preclinical studies on the most potent molecule are under investigation.

Table 1: Effect of compounds on reaction time of mice in Eddy’s hot plate method of analgesia

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>0 hour reaction time mean±SEM (in sec)</th>
<th>1 hour Reaction time mean±SEM (in sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>42.00 ±0.40</td>
<td>59.00 ±0.40</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>44.75 ±0.920</td>
<td>99.75 ±10.16</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>52.75 ±0.900</td>
<td>107.8 ±17.90</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>41.25 ±0.263</td>
<td>75.25 ±10.56</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>57.00 ±0.442</td>
<td>97.00 ±05.14</td>
</tr>
<tr>
<td>6</td>
<td>1f</td>
<td>58.50 ±09.58</td>
<td>124.8 ±16.59</td>
</tr>
<tr>
<td>7</td>
<td>1g</td>
<td>37.50 ±04.57</td>
<td>78.00 ±18.06</td>
</tr>
<tr>
<td>8</td>
<td>1i</td>
<td>46.75 ±10.47</td>
<td>71.50 ±08.56</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>63.75 ±08.30</td>
<td>66.75 ±00.71</td>
</tr>
<tr>
<td>10</td>
<td>Pentazocin</td>
<td>53.35 ±03.08</td>
<td>143.3 ±06.68</td>
</tr>
</tbody>
</table>

***- potent activity, **- good activity, ns- less or no activity.

Fig. 2: Graph showing effect of compounds on reaction time

Table 2: Effect of compounds in percentage of reduction in Paw volume of rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effect of drugs in % of reduction in Paw volume of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hour</td>
</tr>
<tr>
<td>1a</td>
<td>52.95</td>
</tr>
<tr>
<td>1b</td>
<td>43.53</td>
</tr>
<tr>
<td>1c</td>
<td>23.90</td>
</tr>
<tr>
<td>1d</td>
<td>100.00</td>
</tr>
<tr>
<td>1e</td>
<td>11.50</td>
</tr>
<tr>
<td>1f</td>
<td>8.33</td>
</tr>
<tr>
<td>1g</td>
<td>38.33</td>
</tr>
<tr>
<td>1h</td>
<td>37.50</td>
</tr>
<tr>
<td>1i</td>
<td>43.33</td>
</tr>
<tr>
<td>1j</td>
<td>43.33</td>
</tr>
<tr>
<td>1k</td>
<td>31.83</td>
</tr>
<tr>
<td>Difference</td>
<td>47.50</td>
</tr>
</tbody>
</table>
Table 3: Mean increase in Paw volume of rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean increase in paw volume of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.48 0.33 0.18 0.15</td>
</tr>
<tr>
<td>1b</td>
<td>0.53 0.45 0.28 0.38</td>
</tr>
<tr>
<td>1c</td>
<td>0.50 0.43 0.38 0.48</td>
</tr>
<tr>
<td>1d</td>
<td>0.53 0.38 0.28 0.35</td>
</tr>
<tr>
<td>1e</td>
<td>0.50 0.43 0.30 0.40</td>
</tr>
<tr>
<td>1f</td>
<td>0.53 0.43 0.35 0.45</td>
</tr>
<tr>
<td>1g</td>
<td>0.35 0.25 0.18 0.20</td>
</tr>
<tr>
<td>1h</td>
<td>0.35 0.28 0.20 0.20</td>
</tr>
<tr>
<td>1i</td>
<td>0.33 0.25 0.15 0.18</td>
</tr>
<tr>
<td>1j</td>
<td>0.33 0.20 0.15 0.10</td>
</tr>
<tr>
<td>1k</td>
<td>0.38 0.30 0.20 0.20</td>
</tr>
<tr>
<td>Control</td>
<td>0.58 0.50 0.43 0.58</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>0.30 0.18 0.13 0.10</td>
</tr>
</tbody>
</table>
REFERENCES